CLAIMS

We claim:

1. A composition comprising an enzyme, wherein said enzyme comprises a heterologous functional domain, wherein said heterologous functional domain provides altered functionality in a nucleic acid cleavage assay.

2. The composition of Claim 1, wherein said enzyme comprises a 5' nuclease.

3. The composition of Claim 2, wherein said 5' nuclease comprises a thermostable 5' nuclease.

4. The composition of Claim 1, wherein said enzyme comprises a polymerase.

5. The composition of Claim 4, wherein said polymerase is altered in sequence relative to a naturally occurring sequence of a polymerase such that it exhibits reduced DNA synthetic activity from that of the naturally occurring polymerase.

6. The composition of Claim 4, wherein said polymerase comprises a thermostable polymerase.

7. The composition of Claim 6, wherein said thermostable polymerase comprises a polymerase from a *Thermus* species.

8. The composition of Claim 7, wherein said *Thermus* species is selected from *Thermus aquaticus*, *Thermus flavus*, *Thermus thermophilus*, *Thermus filiformus*, and *Thermus scotoductus*.

9. The composition of Claim 1, wherein said heterologous functional domain

comprises an amino acid sequence that provides an improved nuclease activity in said nucleic acid cleavage assay.

10. The composition of Claim 1, wherein said heterologous functional domain comprises an amino acid sequence that provides an improved substrate binding activity in said nucleic acid cleavage assay.

11. The composition of Claim 1, wherein said heterologous functional domain comprises an amino acid sequence that provides improved background specificity in said nucleic acid cleavage assay.

12. The composition of Claim 1, wherein said heterologous functional domain comprises two or more amino acids from a polymerase domain of a polymerase.

13. The composition of Claim 12, wherein at least one of said two or more amino acids is from a palm region of said polymerase domain.

14. The composition of Claim 12, wherein at least one of said two or more amino acids is from a thumb region of said polymerase domain.

15. The composition of Claim 12, wherein said polymerase comprises *Thermus thermophilus* polymerase.

16. The composition of Claim 12, wherein said two or amino acids from said polymerase domain comprise two or more amino acids from amino acids 300-650 of SEQ ID NO:267.

17. The composition of Claim 1, wherein said enzyme comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 106, 109, 112, 115, 118, 121, 124, 127, 130, 133, 136, 139,

142, 145, 148, 150, 153, 157, 160, 163, 166, 169, 172, 175, 178, 181, 184, 187, 190, 200, 202, 204, 206, 212, 214, 216, 218, 221, 226, 228, 230, 232, 234, 236, 239, 241, 243, 251, 259, 261, 263, and 265.

18. The composition of Claim 1, wherein said nucleic acid cleavage assay comprises cleavage of a DNA member of a substrate containing at least one RNA component.

19. The composition of Claim 1, wherein said nucleic acid cleavage assay comprises an invasive cleavage assay.

20. A composition comprising a nucleic acid encoding the enzyme of Claim 1.

21. The composition of Claim 20, wherein said nucleic acid is selected from the group consisting of SEQ ID NOs:74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 105, 108, 111, 114, 117, 120, 123, 126, 129, 132, 135, 138, 141, 144, 147, 149, 152, 156, 159, 162, 165, 168, 171, 174, 177, 180, 183, 186, 189, 199, 201, 203, 205, 211, 213, 215, 217, 220, 225, 227, 229, 231, 233, 235, 238, 240, 242, 250, 258, 260, 262, and 264.

22. The composition of Claim 20, further comprising an expression vector operably linked to said nucleic acid.

23. A composition comprising a host cell containing the composition of Claim 22.

24. A method for producing an altered enzyme with improved functionality in a nucleic acid cleavage assay comprising:

a) providing an enzyme and a nucleic acid test substrate;

b) introducing a heterologous functional domain into said enzyme to produce an altered enzyme;

c) contacting said altered enzyme with said nucleic acid test substrate to produce cleavage products; and

d) detecting said cleavage products.

25. The method of Claim 24, wherein said introducing a heterologous functional domain comprises mutating one or more amino acids of said enzyme.

26. The method of Claim 24, wherein said introducing a heterologous functional domain into said enzyme comprises adding a functional domain from a protein into said enzyme.

27. The method Claim 26, wherein said adding a functional domain from a protein into said enzyme comprising removing a portion of said enzyme sequence prior to adding said functional domain of said protein.

28. The method of Claim 24, wherein said nucleic acid test substrate comprises a cleavage structure.

29. The method of Claim 28, wherein said cleavage structure comprises an RNA target nucleic acid.

30. The method of Claim 28, wherein said cleavage structure comprises an invasive cleavage structure.

31. The method of Claim 24, wherein said enzyme comprises a 5' nuclease.

32. The method of Claim 31, wherein said 5' nuclease comprises a thermostable 5' nuclease.

33. The method of Claim 24, wherein said enzyme comprises a polymerase.

34. The method of Claim 33, wherein said polymerase is altered in sequence

relative to a naturally occurring sequence of a polymerase such that it exhibits reduced DNA synthetic activity from that of the naturally occurring polymerase.

35. The method of Claim 34, wherein said polymerase comprises a thermostable polymerase.

36. The method of Claim 35, wherein said thermostable polymerase comprises a polymerase from a *Thermus* species.

37. The method of Claim 36, wherein said *Thermus* species is selected from *Thermus aquaticus*, *Thermus flavus*, *Thermus thermophilus*, *Thermus filiformus*, and *Thermus scotoductus*.

38. The method of Claim 24, wherein said heterologous functional domain comprises an amino acid sequence that provides an improved nuclease activity in said nucleic acid cleavage assay.

39. The method of Claim 24, wherein said heterologous functional domain comprises an amino acid sequence that provides an improved substrate binding activity in said nucleic acid cleavage assay.

40. The method of Claim 24, wherein said heterologous functional domain comprises an amino acid sequence that provides improved background specificity in said nucleic acid cleavage assay.

41. The method of Claim 24, wherein said heterologous functional domain comprises two or more amino acids from a polymerase domain of a polymerase.

42. The method of Claim 41, wherein at least one of said two or more amino acids is from a palm region of said polymerase domain.

43. The method of Claim 41, wherein at least one of said two or more amino acids is from a thumb region of said polymerase domain.

44. The method of Claim 41, wherein said polymerase comprises *Thermus thermophilus* polymerase.

45. The method of Claim 41, wherein said two or amino acids from said polymerase domain comprise two or more amino acids from amino acids 300-650 of SEQ ID NO:267.

46. The method of Claim 24, wherein said enzyme comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 106, 109, 112, 115, 118, 121, 124, 127, 130, 133, 136, 139, 142, 145, 148, 150, 153, 157, 160, 163, 166, 169, 172, 175, 178, 181, 184, 187, 190, 200, 202, 204, 206, 212, 214, 216, 218, 221, 226, 228, 230, 232, 234, 236, 239, 241, 243, 251, 259, 261, 263, and 265.

47. The method of Claim 24, wherein said altered enzyme comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 106, 109, 112, 115, 118, 121, 124, 127, 130, 133, 136, 139, 142, 145, 148, 150, 153, 157, 160, 163, 166, 169, 172, 175, 178, 181, 184, 187, 190, 200, 202, 204, 206, 212, 214, 216, 218, 221, 226, 228, 230, 232, 234, 236, 239, 241, 243, 251, 259, 261, 263, and 265.

48. An altered enzyme produced by the method of Claim 24.

49. A kit comprising the altered enzyme of Claim 48.

50. A kit comprising the composition of Claim 1.

51. The kit of Claim 50, further comprising at least one nucleic acid cleavage substrate.

52. The kit of Claim 51, further comprising at least one RNA capable of hybridizing to said at nucleic acid cleavage substrate.

53. The kit of Claim 50, further comprising a labeled oligonucleotide.

54. The kit of Claim 50, further comprising an invasive oligonucleotide.

55. A method for cleaving a nucleic acid comprising:

a) providing:

i) the enzyme of Claim 1; and

ii) a substrate nucleic acid; and

b) exposing said substrate nucleic acid to said enzyme.

56. The method of Claim 55, wherein said exposing said substrate nucleic acid to said enzyme produces at least one cleavage product.

57. The method of Claim 56, further comprising the step of c) detecting said cleavage product.